REMARKS

Applicants have amended claim 18, part c) to remove certain references to "the siNA," the "first strand" and the "second strand," replacing the latter two with the "sense strand" and the "antisense strand," respectively, in order to insure proper antecedent basis. These amendments were merely introduced to correct certain inadvertent errors introduced in prior amendments, placing the present claims in a better condition for appeal without changing the scope. No new matter has been added and Applicants respectfully request entry of these amendments. No other claims have been amended, added, or canceled. As such, claims 18, and 20-33 are pending.

Claim rejections – 35 U.S.C. § 102(e)

Claims 18 and 20-33 remain rejected under 35 U.S.C § 102(e) as being anticipated by Fosnaugh et al. (US 2004/0143732, USSN 10/224,005). Final Office Action, at pages 2. The Final Office Action indicated that the prior Rule 1.132 declaration, submitted on October 31, 2008, was not sufficient to overcome the rejection. Without acquiescing to the Office's contentions, and solely in the interest of advancing prosecution, Applicants submit herewith a new Rule 1.132 Declaration that clearly states that the invention disclosed in the reference applications and claimed in the instant application was derived from Dr. James McSwiggen, who is a named inventor of the instant application. As such, the invention claimed herein is not an invention "by another" according to 35 U.S.C. § 102(e).

This new Rule 1.132 declaration thus obviates the 35 U.S.C. § 102(e) rejections, the withdrawal of which is respectfully requested.

Claim Rejections – 35 U.S.C. § 103(a)

Claims 18 and 20-33 remain rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Elbashir *et al.* (2001, EMBO J., v. 20(23): 6877-88), Monia & Cosert (2000, U.S. Patent 6,033,910), Piekin (1991, Science, v. 253-314-7), and Matulic-Adamic (1999, US 5,998,203). Applicants respectfully traverse for the following reasons.

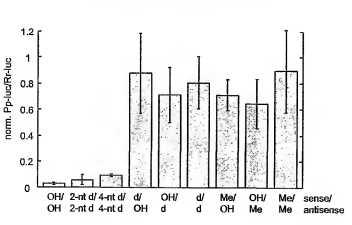
As explained in prior responses and therefore not reiterated here in detail, the references taken together fail to suggest an siRNA molecule that comprises two or more Northern Conformation nucleotides on the sense strand, the antisense strand, or both the strands,

wherein at least two of the modifications are different from each other. However, it is respectfully submitted that the key arguments used by the Office to substantiate the obviousness finding fundamentally lacked basis. For example, the Office stated that "Elbashir teaches that siRNAs with 2'-deoxy nucleotides at the end of siRNA strands were functional and ***suggested that siRNAs with 2'-OMe nucleotides at the end of siRNA strands were also functional,***" (Final Office Action, at page 3), but after an exhaustive search of the Elbashir reference, no suggestion can be found to indicate that the terminally 2'-OMe-modified siRNA were functional. The figure and paragraphs cited by the Office reported siRNA functionality ***only*** when 2'-deoxy modification was applied at the 3'-ends. In fact, every mention of 2'-OMe modification was tied to a ***lack of functionality***.

For ease of comparison, Applicants quote the passage in the last paragraph of page 6881, as cited by the Office:

> To assess the importance of the siRNA ribose residue for RNAi, duplexes with 21-nt siRNAs and 2-nt 3'-overhangs with 2'-deoxy- or 2'-*O*-methyl-modified strands were examined (Figure 4). Substitution of the 2 nt 3'-overhangs by 2'-deoxynucleoties had no effect and even the replacement of two additional ribonucleotides by 2'-deoxyribonucleotides adjacent to the overhangs in the paired region produced significantly active siRNA. Thus, 8 out of 42 nt of a siRNA duplex were replaced by DNA residues without loss of activity. Complete substitution of one or both siRNA strands by 2'-deoxy residues, however, abolished RNAi, as did complete substitution by 2'-*O*-methyl residues.

Figure 4 is also reproduced here, and the constructs of this figure are separately aligned with the description of this figure in the above-quoted paragraph from page 6881:



Sense/antisense	Description in last paragraph, p. 6881	RNAi function, last paragraph, p. 6881
OH/OH	Native, unmodified, or all-RNA duplex	
2-nt d/2-nt d	"[s]ubstitution of the 2nt 3'-overhangs by 2'-deoxynucleotides"	"had no effect"
4-nt d/4-nt d	"even the replacement of two additional ribonucleotides by 2'-deoxyribonucleotides adjacent to the overhangs in the paired region"	"produced significantly active siRNA"
d/OH	"[c]omplete substitution of one … siRNA strand[] by 2'-deoxy residues"	"abolished RNAi"
OH/d	"[c]omplete substitution of one … siRNA strand[] by 2'-deoxy residues"	"abolished RNAi"
d/d	"[c]omplete substitution of … both siRNA strands by 2'-deoxy residues"	"abolished RNAi"
Me/OH	"complete substitution by ***2'-O-methyl*** residues"	"***abolished*** RNAi, as did complete substitution by ***2'-O-methyl*** residues"
OH/***Me***	"complete substitution by ***2'-O-methyl*** residues"	"***abolished*** RNAi, as did complete substitution by ***2'-O-methyl*** residues"
Me/Me	"complete substitution by ***2'-O-methyl*** residues"	"***abolished*** RNAi, as did complete substitution by ***2'-O-methyl*** residues"

(emphasis added, to descriptions related to 2'-O-methyl modification).

The left column of page 6885 merely concludes from the data presented in Figure 4, without providing any additional suggestions:

> 2'-deoxy substitutions of the 2 nt 3'-overhanging ribonucleotides do not affect RNAi, but help to reduce the costs of RNA synthesis and may enhance the RNase resistance of the siRNA duplexes. More extensive 2'-deoxy or 2'-O-methyl modifications reduce the ability of the siRNAs to mediate RNAi, probably by interfering with the protein association for siRNP assembly.

Curiously, the Office appeared to also recognize the fact that Elbashir "does not comment on the results of the 2'-O-methyl-end-modified siRNAs," (Final Office Action, at page 3), which is hardly consistent with the allegation earlier, on the same page of the Final Action, that Elbashir "***suggested*** that siRNAs with 2'-OMe nucleotides at the end of siRNA strands were also functional." Reconsideration and clarification is respectfully requested.

Similarly, The Office asserted that "Monia teaches the use of two different modifications at the ends of antisense oligonucleotides to increase oligonucleotide stability," (Final Office Action, at page 3) without providing basis for such an assertion. It should be noted that antisense molecules of Monia inhibit gene expression by an RNase H mechanism, wherein the enzyme RNase H recognizes and cleaves the mRNA target in an RNA:DNA duplex. *See,*

generally, chapter 6.4 Optimizing Oligonucleoide Drugs, 6.4.2.1., Gapmer Designs, pages 169-170, Antisense Drug Technology, Principles, Strategies, and Applications, Crooke ed., 2nd ed. CRC Press (2006) (copy attached). Thus, an antisense gapmer molecule in its native and unmodified form has at least a DNA region that would form a duplex with the mRNA target, which allows for the recruiting of RNase H and knockdown of gene expression. In a chimeric or "gapmer" construct, such as the ones described in Monia, the ends of the antisense strand, or the "wings" can be modified to enhance stability. Among the gapmers in Monia, however, ***not even one*** uses two different modifications in the wings of a single antisense molecule. *See, e.g.,* Example 5 ([2'-O-Me]-[2'-deoxy]-[2'-O-Me] chmieric phosphorothioate oligonucleotides (column 34, line 24, et seq); [2'O-(2-methoxyethyl)]-[2'-deoxy]-[2'-O-(2-methoxyethyl)] chemiric phosphorothiate oligonucleotides (column 34, line 50, et seq); [2'-O-(2-Methoxyethyl)Phosphodiester]-[2'-deoxy-phosphorothiate]-[2'O-methoxyethyl)phosphodiester] chimeric oligonucleotides (column 34, line 57, et seq); Example 16 (2'-MOE gapmers, wherein the wings are 2'-methoxyethyle nucleotides). Thus there is no basis for the allegation that Monia teaches the use of two different modifications at the ends of antisense oligonucleotides to enhance stability.

As explained above, the DNA stretch of the Monia gapmers should be and would be considered "unmodified" by those skilled in the art. Therefore, each of the Monia gapmers has a single type of Northern conformation modification. However, even assuming, *arguendo,* that an RNA-based antisense molecule was contemplated in the art, the Monia gapmers would still have only one Northern conformation modification per molecule because a 2'-deoxy modification of RNA does not have a Northern conformation.

Moreover, the Office's arguments of obviousness hinges on the notion that those skilled in the art would have been motivated to try known modifications that had been used to enhance nuclease stability in the antisense and ribozyme arts in an siRNA context. But this reasoning does not square with the fact that each of the antisense and ribozyme references cited by the Office disclosed a very large number of modifications that were known to stabilize nucleotides against nucleases, but none provided direction as to which of these to apply in the siRNA context so as to preserve RNAi functionality. It is important to note that the issue here has never been whether the modifications selected by the instant Applicants were known to

enhance nuclease stability, but rather whether there was anything in the art to teach or suggest with specificity which of those known stability-enhancing modifications could be applied to an siRNA without abrogating RNAi functionality.

It is respectfully submitted that the number of known stability-enhancing chemical modifications is huge. For example, Monia describes, in the antisense context, that oligonucleotides can be modified in the backbones or into non-naturally occurring portions "because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target, and increased stability in the presence of nucleases." Column 6, lines 21-24. Preferred modified oligonucleotides backbones include, for example:

> phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

See, paragraph bridging columns 6-7. Moreover, preferred modified oligonucleotide backbones that do not include a phosphorus atom may be:

> backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

See, column 7, lines 22-37. Furthermore, in other preferred oligonucleotide mimetics, both the sugar and the backbone can be replaced with novel groups, such as a PNA. *See,* column 7, lines 48-55. And most preferred embodiments are oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones:

> and in particular $-CH_2-NH-O-CH_2-$, $-CH_2-N(CH_3)-O-CH_2-$ [known as a methylene (methylimino) or MMI backbone], $-CH_2-O-N(CH_3)-CH_2-$, $-CH_2-N(CH_3)-N(CH_3)-CH_2-$ and $-O-N(CH_3)-CH_2-CH_2-$ [wherein the native phosphodiester backbone is represented as $-O-P-O-CH_2-$] of the above referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above referenced U.S. Pat. No. 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

See, paragraph bridging columns 7-8. As to sugar modifications, Monia states that "[p]referred oligonucleotides comprise one of the following at the 2'-position:"

> OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Particularly preferred are $O[(CH_2)_n O]_m CH_3$, $O(CH_2)_n OCH_3$, $O(CH_2)_n NH_2$, $O(CH_2)_n CH_3$, $O(CH_2)_n ONH_2$, and $O(CH_2)_n ON[(CH_2)_n CH_3)]_2$, where n and m are from 1 to about 10.

See, column 8, lines 11-21. Other preferred oligonucleotides may comprise one of the following at the 2' position:

> C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2 CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy (2'-O-$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., Helv. Chim. Acta, 1995, 78, 486-504) i.e., an alkoxyalkoxy group. A further preferred modification includes 2'-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2$ $ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples hereinbelow, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., 2'-O-CH_2-O-CH_2-$N(CH_2)_2$, also described in examples hereinbelow.

See, column 8, lines 22-42. Monia does not stop there, but went on to list even more preferred modifications:

> include 2'-methoxy (2'-O-CH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

See, column 8, lines 43-59. In addition, Monia lists modified nucleobases other than those above, which are said to be "particularly useful for increasing the binding affinity of the oligomeric compounds." *See, e.g.,* paragraph bridging columns 8-9.

It is therefore clear that, if a skilled person in the art were to test all stability-enhancing modifications known in the antisense art ***alone,*** he would have at least all of the ones listed in the

quotes above or otherwise listed in Monia to select from. That is by no means a small number, but ***it gets larger still*** if the skilled person also considers modifications suitable for use in the ribozyme art. For example, the cited Matulic-Adamic reference taught that "modifications protect the enzymatic nucleic acids from exonuclease degradation, ... facilitates efficient uptake ... by cells, ... and help achieve an overall improvement in the efficacy of ribozymes in vitro and in vivo." *See,* Matulic-Adamic, at column 2, lines 44-58. Matulic-Adamic went on to list the suitable modifications as including, but not limited to: (1) terminal modifications (*i.e.,* either a 5'-cap or a 3'-cap); (column 2, lines 47-49); (2) modified bases (column 4, line 62, to column 5, line 5); (3) sugar modifications (column 5, lines 6-22). The terminal modifications alone include a dense list spanning at least an entire column in the printed patent. As such, it can be safely concluded that the skilled person in the art at the time of the present invention was faced with an enormous list of stabilizing chemical modifications but no direction as to which among these could be applied to an siRNA molecule without abrogating activity. Neither Monia, nor Pieken, nor Matulic-Adamic provides that direction, which is not surprising because exogenously introduced short interfering RNAs were not known or experimented with until shortly before the publication of Elbashir.

As explained above, Elbashir indeed provided certain limited directions, for example, as to the applicability of 2'-deoxy modifications at the 3'-terminal nucleotides. Meanwhile, Elbashir provided strong evidence that it was ***highly unpredictable*** in the art at the time which of the known stability-enhancing modifications might be applicable, because even with 2'-deoxy modification, which could be applied to the 3'-terminal nucleotides, more extensive application, which would certainly make the molecule more stable against nucleases, was reported to abrogate RNAi functionality. Another known modification, which was preferred for its ability to enhance stability in antisense molecules, 2'-OMe, was reported by Elbashir to destroy RNAi functionality. Thus, without specific directions as to which of the enormous list of known stability-enhancing chemical modifications to apply, those skilled in the art would need to test each, in every possible pattern, before knowing how RNAi activity might be affected.

The instant claims recite siRNA duplexes that comprise at least two different Northern conformation modifications in a single molecule. With the added limitation of at least two different Northern conformation modifications, the already low predictability between RNAi

function and a single modification becomes exponentially lower, which means even more specific directions or suggestions for the claimed invention must be present in the prior art before such claims can be found obvious. But there was simply nothing in the art to suggest picking the claimed modifications, let alone combining them onto a single siRNA molecule without destroying RNAi functionality. The Office's selection of the particular claimed modifications from the many more suitable or even preferred ones listed in the cited references ***can only be*** the result of hindsight in view of what was disclosed and specifically claimed by the instant Applicants. It has long been established that hindsight is an improper basis for an obviousness finding, therefore, the instant claims are not *prima facie* obvious and Applicants respectfully request withdrawal of the rejections.

Conclusion

In view of the foregoing, Applicants respectfully submit the pending claims are in condition for allowance but for the residual provisional double-patenting issues. If the Examiner believes a telephone conference would expedite prosecution of this application, she is urged to telephone the undersigned at the telephone number below.

Respectfully submitted,

Merck & Co.

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